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16. ABSTRACT

Water quality criteria for toxicity of metal-ligand species to aquatic life are investigated by computer modeling. The speciation calculations are performed using REDEQL2. Correlations of toxicity to concentrations (or activities) of particular species or types of species are discussed with respect to three laboratory studies on zinc and copper.

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Relationships of Activities of Metal-Ligand Species to Aquatic Toxicity

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Much has been published concerning toxicity of metals to aquatic life although only during the past decade have there been interpretations of the toxicity data in terms of the relative toxicity of particular speciation forms, e.g., Cu^{2+}, $CuOH^{+}$, $CuCO_3^o$ (1-7). The specific objective of this paper is to illustrate the use of Factor Analysis in discriminating between toxic and non-toxic species. The procedure to be followed is: determination of equilibrium aqueous speciation, calculation of appropriate factors, correlation of toxicity with these factors, and interpretation of the correlation analysis in terms of particular species activities.

The analysis of toxicity data in terms of speciation products is a difficult task since the variables, species activities, usually are numerous and often are interrelated. Factor Analysis allows one to determine a small number of statistically independent, linear combinations of activities (factors). Correlation of these combinations (factors) with toxicity allows discrimination to be made between toxic and non-toxic species.

Conclusions drawn from a statistical study of this type are only as valid as the data upon which the study is based. The published toxicity study (Andrew, Biesinger, and Glass (2)) which we use for illustration is scientifically sound, however, the number of experimental points and limited ranges of some of the experimental variables do restrict the ability to discriminate between species.

Andrew *et al.* (2) studied the effects of carbonate, orthophosphate, and pyrophosphate on the toxicity of copper(II) to *Daphnia magna* at constant pH and total hardness. They reported mortality rates and reciprocal survival times to be directly correlated with cupric and copper-hydroxo ion activities as determined by equilibrium calculations. They also found toxicity to be negatively related to activities of soluble copper carbonate ($CuCO_3^o$), and independent of total or dissolved copper concentration. Data for their set of experiments involving addition of orthophosphate are included in Table I but were not used in some

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of our later calculations due to uncertainty as to the stability constants for phosphate complexes. Calculations when all points were used are referred to as 30 point and those in which the orthophosphate points are excluded are referred to as 26 point. The calculations described below are of two types; first, recalculation of metal-ligand speciation and comparison with previous results, and secondly, interpretation of the toxicity data in terms of speciation using factor analysis and multiple regression. The speciation calculations were performed using REDEQL2 (8) and SPSS (9) was used for factor analysis and multiple regression.

Sources of Data and Limitations

Stability Constants. The choice of equilibria to include and the accuracy of the related stability constants have, of course, a major effect upon the predicted speciation and upon inferences drawn from correlations of activities of the species with toxicity. A measure of the effects of limited accuracy in the stability constants on predicted speciation concentrations can be obtained through repetitive calculations on a system while allowing for small, random variations in the stability constants. Such calculations have been carried out using a three-metal, three-ligand model (31 complexes) with concentrations ranging from 1 mM to 0.01 μM. Random variations of 0.05 to -0.05 units in the mantissas of the pK's led to concentration changes ranging from 6% to 30% with a mean change of 14%. In general, the percentage changes were greatest for the species whose concentrations were small fractions of the total concentration. Use of smaller random variations of 0.01 to -0.01 units produced proportionately smaller changes, about 1/5 as great.

Toxicity Studies. It is difficult to find published toxicity studies which are well documented and which also contain a sufficient number of data points to allow meaningful statistical analyses. Minimal necessary documentation requires pH, alkalinity, measured concentrations of the particular metals or ligands under study, and a complete analytical background analysis. This presumes of course that: equilibria involving the background species are important and will be considered; total inorganic carbon will be derived from alkalinity and knowledge of other acids present; and the nominal added metal or ligand does not necessarily equal the measured dissolved metal or ligand.

With regard to including all equilibria, for the experiment referred to in line 7 of Table I, the calculated activity of $CuSO_4^o$ is twice that of $Cu_2(OH)_2^{2+}$ and five times that of $Cu(OH)_3^-$. The relative magnitudes of these activities lead one to believe that the amount of copper combined with sulfate and other ligands must be taken into account if calculated activities of species such as $Cu(OH)_3^-$ and $Cu_2(OH)_2^{2+}$ are to be meaningful.

With regard to calculation of total inorganic carbon (TIC), knowledge of the analytical background and the alkalinity allow the calculation of inorganic carbon from (for alkalinity expressed as mg ℓ^{-1} $CaCO_3$)

$$TIC = \frac{1+10^{-pH+6.35}+10^{-10.33+pH}}{(1+2x10^{-10.33+pH})} \left(\frac{Alk}{50,000} - 10^{-14+pH} + 10^{-9.5} \right) \quad (1)$$

when other acids are not present, with appropriate adjustments when other acids are present. Empirical equations expressing TIC in terms of pH and hardness have been used but seem not to be satisfactory over a wide range of pH (10).

With regard to calculated versus measured amounts of metals, in the work of Andrew, Biesinger and Glass (2), the measured dissolved copper varied between 53 and 100% of the nominal copper added to the system (see Table I) and their data is representative of several other published reports when like comparisons are made.

Analysis of Data

The issue to be addressed in a subsequent section is the attribution of the toxicity of copper in aquatic systems to particular speciation forms, e.g., Cu^{2+}, $Cu_m(OH)_n^{2m-n}$, $Cu_j(CO_3)_k^{2j-2k}$. Analysis of published experimental data in this area normally is difficult for several reasons: statistically small numbers of data points in relation to the number of variables, lack of independence of the variables with correlation coefficients often of the order of 0.8 or 0.9 (see Table II), and small ranges and scatter of points due to the usual experimental practice of varying as few parameters as possible in a particular run with the intent of determining bivariate relationships.

In the first set of experiments of Andrew *et al.* (2), for example, pH, hardness and total alkalinity are held constant while copper is added to solutions with fixed added amounts of $NaHCO_3$, or Na_2HPO_4, or $Na_4P_2O_7$, in addition to copper added to the background, with no subset of experiments including more than six cases. In our speciation calculations related to this set of nineteen experiments, some twenty-five complexes of copper including three with carbonate, two with phosphate, six with hydroxide, and six with pyrophosphate are involved.

It is possible to create new variables, e.g., as linear combinations of the activities, intended to characterize a particular aspect of the system and then interpret the data in terms of this smaller set of new variables. One might, for example, use the sum of the activities of Cu^{2+}, $CuOH^+$ and $Cu_2(OH)_2^{2+}$ as a variable rather than the three species separately (10). In this way a large number of bivariate correlations can be made, but

TABLE I

Calculated Equilibrium Values for Activities of Selected Species Involved in the Andrew's Experiments. Values shown are the negative logarithms of the activities

Nominal Added Cu(μM)	Measured Dissolved Cu(μM)	Cu^{2+}	$CuOH^{+}$	$Cu_2(OH)_2^{2+}$	$Cu(OH)_2^{o}$	$Cu(OH)_3^{-}$	$Cu(OH)_4^{2-}$	$CuCO_3^{o}$	$Cu(CO_3)_2^{2-}$
				Lake Superior Water Only, pH 7.85-8.05					
Control	~0.02	10.06	9.80	14.57	7.85	12.99	17.84	8.77	11.06
0.20	0.20	9.00	8.72	12.41	6.75	11.87	16.71	7.69	9.96
0.32	0.33	8.78	8.50	11.97	6.54	11.65	16.49	7.48	9.74
0.51	0.41	8.69	8.41	11.79	6.44	11.56	16.40	7.38	9.64
0.80	0.61	8.51	8.24	11.44	6.27	11.39	16.22	7.21	9.47
1.27	1.02	8.29	8.01	10.99	6.05	11.16	16.00	6.99	9.25
2.01	1.54	8.11	7.83	10.64	5.87	10.98	15.82	6.81	9.07
				Lake Superior Water + 48 μM $NaHCO_3$, pH 7.85-8.05					
Control	~0.02	10.11	9.83	14.62	7.85	12.97	17.80	8.77	11.01
0.80	0.60	8.50	8.24	11.44	6.28	11.41	16.26	7.19	9.44
1.27	0.98	8.27	8.02	11.00	6.07	11.21	16.07	6.97	9.23
2.01	1.45	8.08	7.83	10.64	5.90	11.05	15.92	6.78	9.06
				Lake Superior Water + 210 μM Na_2HPO_4, pH 7.85-8.05					
Control	~0.02	10.12	9.84	14.64	7.86	12.98	17.82	8.71	10.88
0.80	0.54	8.58	8.30	11.57	6.34	11.46	16.31	7.18	9.35
1.27	0.76	8.42	8.15	11.27	6.19	11.32	16.16	7.03	9.20
2.01	1.07	8.27	8.00	10.97	6.04	11.17	16.03	6.88	9.06

TABLE I Cont'd.

Nominal Added Cu(μM)	Measured Dissolved Cu(μM)	Cu^{2+}	$CuOH^{+}$	$Cu_2(OH)_2^{2+}$	$Cu(OH)_2^{o}$	$Cu(OH)_3^{-}$	$Cu(OH)_4^{2-}$	$CuCO_3^{o}$	$Cu(CO_3)_2^{2-}$
Lake Superior Water + 110 μM $Na_4P_2O_7$, pH 7.85-8.05									
Control	0.02	10.85	10.54	16.06	8.55	13.64	18.45	9.51	11.74
0.80	0.77	9.16	8.86	12.69	6.87	11.97	16.78	7.83	10.06
1.27	1.24	8.95	8.65	12.28	6.67	11.76	16.58	7.62	9.86
2.01	1.95	8.74	8.45	11.86	6.46	11.57	16.39	7.41	9.66
Lake Superior Water + (0,1,2,4,10 mM) added $NaHCO_3$, pH 7.3-7.5									
5	3.38	6.77	7.07	9.01	5.67	11.35	16.76	6.03	8.86
5	3.82	6.83	7.12	9.21	5.72	11.40	16.80	5.78	8.29
5	4.13	6.88	7.17	9.31	5.77	11.44	16.85	5.66	7.99
5	4.21	7.01	7.29	9.56	5.89	11.56	16.97	5.57	7.67
5	4.37	7.24	7.52	10.02	6.12	11.78	17.20	5.47	7.23
Lake Superior Water + (0,2.5,5.0,10.0,50.0,500 μM) added $Na_4P_2O_7$, pH 7.4-7.6									
5	***	6.75	6.96	8.88	5.47	11.07	16.39	5.93	8.67
5	***	6.90	7.09	9.15	5.59	11.18	16.48	6.06	8.79
5	***	7.03	7.21	9.39	5.70	11.27	16.56	6.18	8.89
5	***	7.24	7.40	9.76	5.86	11.42	16.69	6.36	9.06
5	***	7.89	8.04	11.05	6.50	12.04	17.30	7.00	9.67
5	***	9.75	9.91	14.78	8.38	13.92	19.21	8.80	11.41

*** Values Assumed To Be 5.

TABLE II

Correlation Coefficients Between the Activities of Copper Species in the 26 point Calculations

	Cu^{2+}	$CuOH^{+}$	$Cu_2(OH)_2^{2+}$	$Cu(OH)_2^{o}$	$Cu(OH)_3^{-}$	$Cu(OH)_4^{2-}$	$CuCO_3^{o}$	$Cu(CO_3)_2^{2-}$
Cu^{2+}		0.99	0.94	0.89	0.34	-0.21	0.65	0.22
$CuOH^{+}$	0.99		0.96	0.95	0.45	-0.12	0.60	0.17
$Cu_2(OH)_2^{2+}$	0.94	0.96		0.91	0.41	-0.14	0.46	0.05
$Cu(OH)_2^{o}$	0.89	0.95	0.91		0.70	0.18	0.47	0.07
$Cu(OH)_3^{-}$	0.34	0.45	0.41	0.70		0.83	0.06	-0.12
$Cu(OH)_4^{2-}$	-0.21	-0.13	-0.14	0.18	0.83		-0.26	-0.21
$CuCO_3^{o}$	0.65	0.60	0.46	0.47	0.06	-0.26		0.84
$Cu(CO_3)_2^{2-}$	0.22	0.17	0.05	0.07	-0.12	-0.21	0.84	

direct comparisons between the new variables are difficult since interrelationships of the new variables are not clear and they may be correlated with each other.

What is desired is a logical, systematic procedure to generate a small set of uncorrelated variables which allow direct comparison, make chemical sense, and are amenable to easy interpretation. A mathematical procedure which meets these criteria is the use of factor analysis (9, 11, 12, 13) to generate statistically independent new variables (factors) followed by correlation of toxicity with the generated factors.

Factor analysis has two general uses; to see whether underlying patterns of relationships exist among sets of variables, and to rearrange or reduce the data (variables) to a smaller set of factors or components that may be taken as primary or source variables. One often is cautioned about the first use, employment of factor analytic methods to determine relationships among the variables, in that spurious patterns may be indicated. In our applications, however, the desired patterns, i.e. combinations of activities, normally will be known or can be predicted and the use of factor analysis will be to generate a reduced set of statistically independent components (factors), e.g. three factors replacing eight individual activities. In cases where the patterns are not clear, one retains the criterion that the results must be chemically rational and may reject those that fail to meet this criterion. These factors then are used in multiple regression analyses to attempt to identify toxic species.

Toxicity of Copper to Aquatic Forms

General. A variety of conclusions have been published in recent years on the relative toxicity of free copper ion (Cu^{2+}), hydroxo copper complexes, ($CuOH^{+}$, $Cu(OH)_2^o$, $Cu(OH)_3^-$, $Cu(OH)_4^{2-}$, $Cu_2(OH)_2^{2+}$), and carbanato copper complexes ($CuCO_3^o$, $Cu(CO_3)_2^{2-}$). The statements have been, in general, qualitative in nature rather than quantitative. Shaw and Brown (14) concluded that $CuCO_3^o$ is as toxic as Cu^{2+}; Pagenkopf *et al.* (15) find the major toxic species to be Cu^{2+} with a possible contribution from $CuOH^{+}$: Andrew *et al.* (2) state that copper toxicity is directly related only to the activities of Cu^{2+}, $CuOH^{+}$, and $Cu_2(OH)_2^{2+}$; Chakoumakos *et al.* (16) report Cu^{2+}, $CuOH^{+}$, $Cu(OH)_2^o$ and $Cu_2(OH)_2^{2+}$ to be toxic forms and found $CuHCO_3^{+}$, $CuCO_3^o$ and $Cu(CO_3)_2^{2-}$ not toxic under their conditions; Hawarth and Sprague (10) found the smoothest response surface, of those tried, for copper toxicity to be generated by $[Cu^{2+}]$ + $[CuOH^{+}]$ + $[Cu_2(OH)_2^{2+}]$ and, on the basis of this, claim these three to be the toxic forms and, on the basis on non-smooth response surfaces, that $Cu(OH)_2^o$ and the carbonato copper species are not toxic.

Most of the experiments reported upon in the above referenced papers involved systems with pH in range 6.5-8.5, hardness up to 350mg ℓ^{-1} as $CaCO_3$, initial alkalinity as high as 300mg ℓ^{-1} as $CaCO_3$, and total copper up to 5mg ℓ^{-1}.

In the following sections, factor analysis and multiple regression are used in an attempt to determine the relative toxicity of the copper species discussed above. The activities of eight species (Cu^{2+}, $CuOH^{+}$, $Cu_2(OH)_2^{2+}$, $Cu(OH)_2^{o}$, $Cu(OH)_3^{-}$, $Cu(OH)_4^{2-}$, $CuCO_3^{o}$, $Cu(CO_3)_2^{2-}$) are included in the calculations.

Factor Determination. One is strongly tempted to generate "universal" factors for the species of interest, valid over typical ranges of the parameters involved. The problem is that the points for a given experiment are unlikely to be representative of the points used to generate the "universal" factors and the factors lose their statistical independence. One has little choice then, when using published data, but to use the experimental points directly in the determination of the factors as well as in the correlations and we have done so in the systems reported here although we later discuss the structure of the experimental design which would be most useful to this type of analysis. The quality of species separation obtained through use of previously published experimental points is good however, since the parameter selections made by the experimenters often were for the purpose of studying toxicity as a function of speciation.

A comparison of factors determined with 100 random points and with 30 and 26 experimental points can be made from the data shown in Table III. A short description of the data may be useful to those unfamiliar with factor analysis. The values shown are "factor loadings" for the species on the factors, e.g. the top line under the 100 random point heading states that

$$\text{activity}(Cu^{2+}) = 0.88\times\text{Factor 1} - 0.19\times\text{Factor 2} + 0.27\times\text{Factor 3} \quad (2)$$

and the activity of Cu^{2+} can be calculated from values of the factors. The "factor loadings" are correlation coefficients and the relative importance of the factors in determining the activity of Cu^{2+} is given as the square of the coefficients, implying that Factor 1 is sufficient to explain 77% of the variance in the activity of Cu^{2+}, Factor 2 explains 4%, and Factor 3 explains 7%. The fact that these add up to 0.88 rather than 1.00 states that these three factors are sufficient to explain only 88% of the variation in the activity of Cu^{2+} in the 100 points. In comparison, three factors are sufficient to explain 98% of the variation of the activity of Cu^{2+} in Andrew's 30 point and 26 point cases.

The actual activities of the species are not used in the determination of factors, but instead Kaiser normalized values of the activities are used,

TABLE III

Three-Factor Case for Random Points and the Andrew *et al.* Experiments

Varimax Rotated Factor Matrices After Rotation with Kaiser Normalization

	100 Random Points			Andrew's 30 Points			Andrew's 26 Points		
Species	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Cu^{2+}	0.88	-0.19	0.27	0.96	-0.05	0.24	0.96	-0.05	0.24
$CuOH^{+1}$	0.97	0.06	0.22	0.98	0.05	0.20	0.98	0.05	0.19
$Cu_2(OH)_2^{2+}$	0.96	0.04	-0.03	0.97	***	0.05	0.97	0.02	0.04
$Cu(OH)_2^0$	0.59	0.73	0.08	0.92	0.36	0.11	0.92	0.35	0.11
$Cu(OH)_3^-$	0.04	1.00	-0.01	0.39	0.92	-0.04	0.41	0.91	-0.04
$Cu(OH)_4^{2-}$	-0.12	0.95	0.02	-0.16	0.97	-0.13	-0.15	0.97	-0.13
$CuCO_3^0$	0.38	0.04	0.88	0.46	-0.07	0.87	0.45	-0.09	0.87
$Cu(CO_3)_2^{2-}$	-0.18	0.70	0.56	0.01	-0.08	0.95	***	-0.08	0.95
Percent of overall variance in the points accounted for by the 3 factors	48.6	39.0	12.4	57.6	27.5	14.9	57.5	27.5	14.9

*** Value less than precision shown

$$X' = \frac{X - \bar{X}}{\sigma_X} \qquad (3)$$

where X is the experimental value, X' is the normalized value, $\bar{X}$ the mean of the X and σ_X the standard deviation of the X. Kaiser normalized variables have nominal means of zero and standard deviations of one. Equation 2 should be written instead as

$$A'(Cu^{2+}) = 0.88\ F_1 - 0.19\ F_2 + 0.27\ F_3 \qquad (2')$$

where $A'(Cu^{2+})$ is the Kaiser normalized activity of Cu^{2+} and the factors are Kaiser normalized variables.

From known values of activities, one can calculate the value of the factor (factor score) *via* factor score coefficients,

$$F_1 = c_{11}X'_1 + c_{12}X'_2 + \ldots + c_{1n}X'_n \qquad (4)$$

where the c_{pq} are the factor score coefficients and the X'_q are the normalized values of the activites. The means and standard deviations of the activities of the species, plus the factor score coefficients for the 26 point case are given in Table IV. The second factor would be written

$$F_2 = -1.09\ A'(Cu^{2+}) + 7.19\ A'(CuOH^{+}) + \ldots \qquad (5)$$

TABLE IV

Means, Standard Deviations, and Factor Score Coefficients for Andrew's 26 Points with Three Factors. (Means and Standard Deviation in Molarities)

Species	Mean Activity	Standard Deviation	Factor Score Coefficients		
			Factor 1	Factor 2	Factor 3
Cu^{2+}	4.28×10^{-8}	6.10×10^{-8}	-19.55	-1.09	3.11
$CuOH^{+}$	2.65×10^{-8}	3.33×10^{-8}	48.73	7.19	-7.89
$Cu_2(OH)_2^{2+}$	1.91×10^{-10}	3.36×10^{-10}	0.23	0.29	0.06
$Cu(OH)_2^{o}$	9.39×10^{-7}	9.03×10^{-7}	-38.18	-14.37	4.95
$Cu(OH)_3^{-}$	3.62×10^{-12}	2.85×10^{-12}	16.76	14.23	-1.05
$Cu(OH)_4^{2-}$	3.62×10^{-17}	3.81×10^{-17}	-5.61	-7.48	-0.09
$CuCO_3^{o}$	5.75×10^{-7}	9.26×10^{-7}	-1.34	-0.01	1.14
$Cu(CO_3)_2^{2-}$	4.14×10^{-9}	1.20×10^{-8}	0.49	0.05	0.18

Using multiple regression, biological response (BR) can then be correlated with the factors (factor scores) as

$$BR = aF_1 + bF_2 + cF_3 + \ldots \qquad (6)$$

thereby generating a relationship between biological response and activities, e.g. Equations 7 and 8.

The factor score coefficients (c_{pq}) are not correlation coefficients for independent variables and the concept of relative weight of the activities of the species does not hold, i.e. the squares of the coefficients do not give the relative importance of the activities in the factor.

The quality of species separation appears to be better in the 30 and 26 experimental point cases, that is to say that the loadings generally are more restricted to single factors, especially for $Cu(OH)_2^o$ and $Cu(CO_3)_2^{2-}$ although $Cu(OH)_3^-$ and $CuCO_3^o$ are somewhat less well separated (Table III). The boxes drawn are to indicate the principal species dominating a factor, e.g. under Andrew's 30 point case Factor 1 is primarily (Cu^{2+}, $CuOH^+$, $Cu_2(OH)_2^{2+}$, $Cu(OH)_2^o$) with Factor 2 represented well by ($Cu(COH)_3^-$, $Cu(OH)_4^{2-}$) and Factor 3 by ($CuCO_3^o$, $Cu(CO_3)_2^{2-}$). Note in the 100 random point case that $Cu(OH)_2^o$ distributes 35% on Factor 1 and 53% on Factor 2 and $Cu(CO_3)_2^{2-}$ distributes 49% on Factor 2 with 31% on Factor 3, relatively poor separations. An often used rule-of-thumb is that loadings greater than 0.3 should be considered significant (12, p.10). As will be evident from the discussion below on separability of species, the 100 random-point-factors are a more accurate representation of the behavior of the hydroxo complexes over a wide pH range than are the 30 and 26 point factors. The experiments of Andrew *et al.* are restricted to three pH values (7.4, 7.5, 7.95) thereby limiting the relative variation of hydroxo complex activities, creating a set of data more easily represented by three factors and possibly giving the false impression that the separations achieved are better. Further attention has not been given to factors generated from random points due to problems of statistical independence earlier discussed.

Separation of species by factor analysis is limited in several ways. In order of decreasing importance the separability is controlled by: speciation behavior over the ranges of parameters used, experimental point distribution over the ranges of parameters, number of species involved in the calculation, and number of factors determined.

With regard to speciation behavior: in Figure 1 are displayed the speciation curves for the activities of free copper ion and the hydroxo-complexes of copper over a range of pH. Inspection of the curves reveals that $CuOH^+$ and $Cu_2(OH)_2^{2+}$ have the same pattern of behavior over the entire range and cannot be separated, $Cu(OH)_3^-$ and $Cu(OH)_4^{2-}$ are similar enough that separation is not to be expected, and over the range of pH values used by Andrew *et al.* 7.4-8.0, one should not expect separations other than those observed. One might, upon first glance, feel that the forms of the factors are completely described by the correlation coefficients

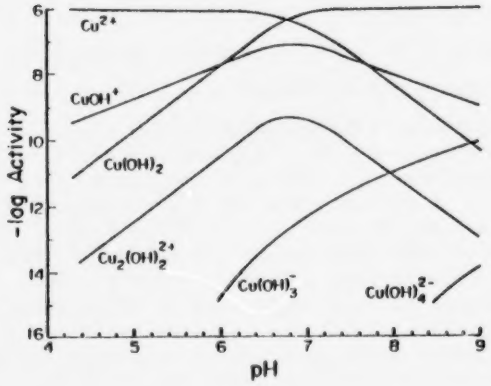

Figure 1. Speciation curves for the activities of free copper ion and the hydroxo complexes of copper vs. pH

given in Table II. The boxes drawn around sets of correlation coefficients correspond to the boxes in Table III and indeed, the largest correlation coefficients lie within the boxes although quite substantial values do lie without. No mathematical manipulation can obviate the data in Table II and of course the factors are derived from the correlation coefficients. The usefulness of factor analysis lies in its ability to determine a small set of statistically independant variables which retain as much information as possible about the system.

With regard to point distribution: it should be clear from Figure 1 that the pH values must be representative of the range 6.0-8.0 for the above discussed separation to be achieved.

With regard to the number of species involved: the number of factors is restricted to no more than one-half the number of variables if convergent, unique values are to be obtained for communalities of the variables (11, p.200), (the communality, of a variable is the amount of the variance of that variable accounted for by the common factors). Therefore, to use a five factor decomposition of a set of species one must have at least 10 species included in the calculation.

With regard to the number of factors: it should be obvious that to obtain a separation of the four groups mentioned later, at least four factors must be used. If other species which have quite different patterns of behavior over the set of points are included in the calculation, sufficient additional factors must be included to account for each speciation pattern or the patterns will smear over the factors.

Addition of more species or factors for Andrew's data would not have proven fruitful due to the limited pH range involved in the experiment.

Finally, after all of the above have been taken into account, it is possible to "rotate" a set of factors with different types of rotation yielding somewhat different species loading (9). If the speciation patterns for the points are not distinct however, rotation of the factors cannot achieve a separation. We have found Varimax rotation, which maximizes the squared loadings of variables in each factor while retaining the orthogonality of the factors to be most useful.

Copper Toxicity to Daphnia Magna

Lake Superior water was used as the dilution water for all experiments by Andrew (2). A copy of the current analytical background for Lake Superior water, Table V, was obtained from the Environmental Research Laboratory-Duluth and was used in our speciation calculations. The water has a total hardness of $\sim$45mg ℓ^{-1} as $CaCO_3$, alkalinity of $\sim$42mg ℓ^{-1} as $CaCO_3$, and a pH from 7.4 to 8.2.

TABLE V

Concentrations used to Simulate the Analytical Background of Lake Superior Water (Molarities)

Calcium	3.26×10^{-4}	Zinc	1.10×10^{-8}
Magnesium	1.10×10^{-4}	Cobalt	3.40×10^{-9}
Sodium	5.60×10^{-5}	Barium	1.00×10^{-7}
Potassium	1.29×10^{-5}	Strontium	1.80×10^{-7}
Cadmium	1.80×10^{-10}	Silver	1.00×10^{-10}
Chromium	3.80×10^{-9}		
Copper	1.60×10^{-8}		
Iron	5.40×10^{-8}	Carbonate	8.81×10^{-4}
Mercury	5.01×10^{-11}	Chloride	3.61×10^{-5}
Methyl Mercury	5.01×10^{-12}	Sulfate	3.65×10^{-5}
Manganese	5.50×10^{-9}	Silicate	4.47×10^{-5}
Nickel	3.40×10^{-8}	Phosphate	3.24×10^{-10}
Lead	4.84×10^{-10}	Nitrate	1.66×10^{-5}

The pH was adjusted in each of their experiments by regulating CO_2-air mixtures bubbled through the solution, e.g. $Na_4P_2O_7$ was added to the solution followed by bubbling sufficient CO_2 through the solution to bring the pH back to 7.95. This process increases the total inorganic carbon. REDEQL2 does allow one to determine the amount of $2H^+ + CO_3^{2-}$ necessary to add to bring the pH back to 7.95. The total carbonate concentrations used in our calculations are listed in Table VI.

TABLE VI

Total Carbonate Values used in Recalculation of Andrew's Data

Andrew's Table	Experiments	Total Carbonate (mM)
2	1 - 7	0.881
	8 - 11	0.929
	12 - 15	1.114
	16 - 19	0.889
3	1	0.935
	2	1.936
	3	2.938
	4	4.932
	5	10.940
4	1 - 4	0.920
	5	0.931
	6	1.119

Speciation Calculations. Although REDEQL2 was used to determine speciation in Andrew's work as well as ours, consider-

able differences exist in predicted equilibrium activities due to modifications which have been made to the program and the associated data base. The major contribution to the differences is the inclusion of more complexes, e.g. $Cu(OH)_2^o$. Complexation by uncharacterized organic ligands and absorption of species or suspended matter were not considered in these speciation calculations. (Both organic complexation and absorption should be less serious problems in this Lake Superior study as compared to a non-laboratory system.) In Table VII some comparative data are listed and in Table I are shown the calculated activities for the free copper ion plus the hydroxo and carbonato complexes.

TABLE VII

Comparisons of Some Calculated Copper Species Activities from this work (M-G) with that of Andrew, *et al* (ABG)

	Calculated Activity of Copper Species (μM)							
Measured dissolved copper (μM)	Cu^{2+}		$CuOH^+$		$Cu(OH)_2^o$		$CuCO_3^o$	
	ABG	M-G	AbG	M-G	ABG*	M-G	ABG	M-G
~0.02	0.001	***	0.001	***	0.014	~0.02	0.002	
0.20	0.011	0.001	0.009	0.002	0.178	0.18	0.020	
0.33	0.016	0.002	0.015	0.003	0.288	0.30	0.033	
0.41	0.020	0.002	0.019	0.004	0.363	0.36	0.042	
0.61	0.031	0.003	0.028	0.006	0.537	0.54	0.062	
1.02	0.034	0.005	0.047	0.010	0.891	0.89	0.102	
1.54	0.076	0.008	0.071	0.015	1.349	1.35	0.155	

* $Cu(OH)_2^o$ was not considered in ABG calculations
*** Value less than precision shown

Single Ion Correlations with Toxicity. The data given in the first fifteen cases, excluding those with added Na_2HPO_4, were used to determine correlations of inverse median survival time with species activity for free Cu^{2+} and the seven listed complexes. The values for r ranged from 0.97 to 0.98 with significance 0.00001 for all species but one, $Cu_2(OH)_2^{2+}$, which exhibited an r of 0.95.

Five of the points (1, 8, 9, 10, 12 excluding Na_2HPO_4 points) are "zero-points", i.e. median survival time exceeded the life of the experiment, and a better test of the hypothesis of toxicity due to copper species results from discarding the zero-points. When this is done, the correlation coefficients decrease by 0.01 or less and the level of significance remains at the 10^{-5} level.

In Figure 2 are shown the inverse median survival time versus activity plots for Cu^{2+} and $CuCO_3^o$, both for the 15 point cases and the 10 point cases.

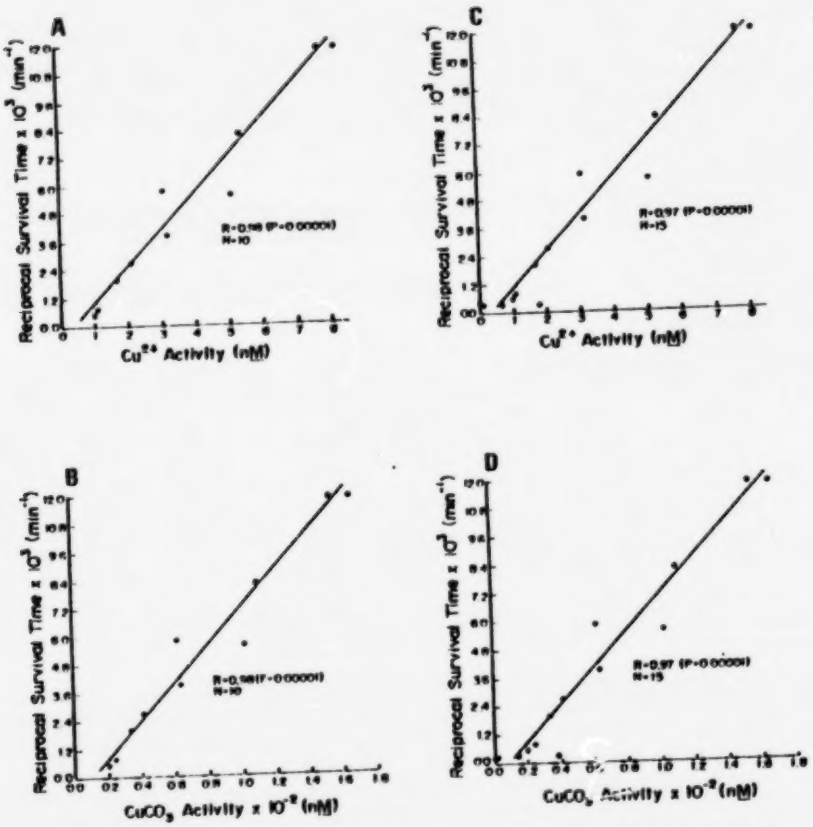

Figure 2. Reciprocal median survival time vs. activity for Cu^{2+}: (A) 10 point case, (C) 15 point case; for $CuCO_3^o$: (B) 10 point case, (D) 15 point case

Factor Analysis and Multiple Regression. Factor scores for the 26 and 30 point cases, generated using the factor score coefficients in Table IV, were correlated with inverse median survival time, t^{-1}.

The 26-point case yielded the following relationship

$$\frac{1}{t} = 0.0046\ F_1 + 0.0027\ F_2 - 0.00013\ F_3 + 0.0061 \qquad (7)$$

while the 30-point case yielded

$$\frac{1}{t} = 0.0051\ F_1 + 0.0024\ F_2 - 0.00011\ F_3 + 0.0055 \qquad (8)$$

Standard errors, 95% confidence intervals, and levels of significance are tabulated in Table VIII for the coefficients in Equations 7 and 8.

TABLE VIII

Standard Errors, 96% Confidence Intervals, Levels of Significance and Changes in R^2 for the 26-Point and 30-Point Regression Equations

26-Point Case	Coefficient x 10^3	Standard Error x 10^3	95% Confidence Interval x 10^3	Level of Significance	Change in R^2
F_1	4.6	0.57	(3.4,5.8)	<0.001	0.70
F_2	2.7	0.60	(1.4,3.9)	<0.001	0.15
F_3	-0.13	0.62	(-1.4,1.2)	0.84	***
Constant	6.1	0.60	(4.9,7.4)		
30-Point Case					
F_1	5.1	0.46	(4.1,6.1)	<0.001	0.61
F_2	2.4	0.46	(1.4,3.4)	<0.001	0.18
F_3	-0.11	0.47	(-1.1,0.86	0.82	***
Constant	5.5	0.46	(4.6,6.5)		

*** Value less than precision shown

The constant terms are the mean values of inverse median survival time in each case since the factor scores (F_1,F_2, F_3) have nominal means of zero and standard deviations of 1.

The relative contributions to the variance in 1/t for each factor are given in the last column of Table VIII. The values are the squares of the correlation coefficients for each variable in Equations 7 and 8 since the factors are orthogonal variables. The sums of contributions are 85% in the 30-point case and 80% in the 26-point case. That these values are less than 100% states that the three factors are sufficient to account for 85% and 80%, respectively, of the variance in 1/t.

Although single ion correlations differ substantially between

the 26 and 30 point cases (r decreases by about 0.1 to 0.2 units in going from 26 to 30 points), the derived factors and multiple regression coefficients are in good agreement for the two calculations.

Conclusions for *Daphnia magna* - Needed Experiments. Conclusions drawn from the above data treatment are necessarily tentative due to the quality of species separation, the numbers of data points involved, and the narrow pH range involved in the calculations.

If the numbers were accepted at face value, the data indicate that: the species involved account for 80-85% of the toxicity, the carbonato complexes do not contribute to toxicity, the anionic hydroxo copper complexes contribute 15-18% to the total toxicity, and the free copper ion and/or the neutral and cationic hydroxo complexes of copper are responsible for 60-70% of the toxicity of copper to aquatic life.

If the carbonato complexes are accepted as being non-toxic, a set of experiments are needed in which the toxicity of copper to Daphnia magna is measured at equal intervals over the pH range 6.0-8.0. The speciation curves shown in Figure 1 would predict that this set of experiments plus inclusion of all copper complexes involved in the speciation calculations should allow separation of the species of concern into four groups, (Cu^{2+}), ($Cu(OH)_2^o$), ($CuOH^+$,$Cu_2(OH)_2^{2+}$), ($Cu(OH)_3^-$, $Cu(OH)_4^{2-}$) and a determination of the relative toxicity of these species. Underlying these statements is the assumption that the mechanism of interaction of copper at the environmental interface remains the same over this pH range.

Sensitivity of Conclusions to Errors in Stability Constants

As one measure of the sensitivity of the conclusions concerning toxic species to errors in the input data, the 26 point calculations have been repeated with a drastic change in the value of the logarithm one stability constant, β for $Cu(OH)_2^o$. The correct value of β_2 for $Cu(OH)_2^o$ has been the subject of much discussion. A value of 14.3 was used in the species calculations in the first part of this paper. Log β_2 was changed to 11.8 and the speciation calculation was repeated with all other input data held constant. Since $Cu(OH)_2^o$ was the dominant copper species in the first calculation, the effect of changing the stability constant by a factor of $\sim$300 has a marked effect on the calculated activities (Table VII and Table IX). The resulting activities were used as input for factor analysis. In Table X are shown the factors for the 26 point case. Decreasing the stability constant of $Cu(OH)_2^o$ $\sim$300 fold causes a shift in the loading of $Cu(OH)_2^o$ from Factor 1 to Factor 2, of $CuCO_3^o$ from Factor 3 to Factor 1 and of $Cu(OH)_3^-$ from Factor 1 to Factor 2, with minimal changes for the other species. Examination

TABLE IX

Calculated Copper Species Activities with log (β_2) for $Cu(OH)_2$ of 11.8

Measured dissolved copper (μM)	Activity of Copper Species (μM)			
	Cu^{2+}	$CuOH^+$	$Cu(OH)_2^o$	$CuCO_3^o$
~0.02	0.001	0.001	***	0.013
0.20	0.008	0.016	0.004	0.166
0.33	0.014	0.026	0.008	0.275
0.41	0.017	0.032	0.010	0.347
0.61	0.026	0.048	0.014	0.513
1.02	0.043	0.079	0.023	0.851
1.54	0.064	0.120	0.035	1.288

*** Value less than precision shown

of speciation curves versus pH, Figure 1, would lead one to predict these shifts. It should be noted that the species that shifted from one factor to another have moderate or high loadings in two factors.

TABLE X

Comparison of the Varimax Rotated Factor Matrices for Three-Factor 26-Point Case

Species	Factor 1		Factor 2		Factor 3	
	A	B	A	B	A	B
Cu^{2+}	0.96	0.97	-0.05	-0.05	0.24	0.17
$CuOH^+$	0.98	0.97	0.05	0.23	0.19	0.12
$Cu_2(OH)_2^{2+}$	0.97	0.94	0.02	0.07	0.04	0.01
$Cu(OH)_2^o$	0.92	0.61	0.35	0.79	0.11	0.02
$Cu(OH)_3^-$	0.41	0.10	0.91	0.99	-0.04	-0.04
$Cu(OH)_4^{2-}$	-0.15	-0.10	0.97	0.99	-0.13	-0.05
$CuCO_3^o$	0.45	0.60	0.09	-0.01	0.87	0.77
$Cu(CO_3)_2^{2-}$	***	-0.02	0.08	-0.05	0.95	0.91

*** Value less than precision shown

A = Calculations with $\beta_2 = 10^{14.3}$ for $Cu(OH)_2^o$

B = Calculations with $\beta_2 = 10^{11.8}$ for $Cu(OH)_2^o$

Multiple regression analysis utilizing factor scores for the above factors gives the following:

$$\frac{1}{t} = 0.0052F_1 + 0.0021\ F_2 + 0.00084\ F_3 + 0.0061 \qquad (9)$$

with changes in R^2 in the regression analysis due to F_1, F_2, and F_3 of 0.68, 0.10, and 0.02, respectively. As before, the Cu species involved account for 80% of the toxicity. Since only the carbonato species dominate the third factor (which accounts for 2% of the variance in t^{-1}), the carbonato complexes contribute, at worst, only slightly to the toxicity. The anionic hydroxo copper complexes, along with $Cu(OH)_2^0$, loaded in factor 2, can contribute only moderately to copper toxicity. Free copper ion and/or the cationic hydroxo complexes, along with $Cu(OH)_2^0$, again are predicted to be responsible for the major portion of the toxicity of copper to aquatic life.

Summary

A procedure is developed through which the relative toxicity of metal speciation products can be determined. The procedure is illustrated utilizing data from a published study on the toxicity of copper to *Daphnia magna* and tentative conclusions are drawn with respect to the relative toxicity of certain combinations of species. The experiments necessary to obtain a better description of the relative toxicity of copper species to toxicity are described.

Some limitations of the procedure are discussed. A thorough error analysis of the procedure is not given but is in progress and will be the subject of a later paper.

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Abstract

The use of factor analysis and multiple regression in determining relative toxicities of species is described. Procedures are illustrated using the data of Andrew, Biesinger and Glass from a study of toxicity of copper to *Daphnia magna* following a recalculation of the equilibrium speciation of copper. The results yield tentative conclusions that the carbonato copper complexes are not toxic, the anionic hydroxo copper complexes contribute 15-18% to the toxicity of copper, and that free copper and/or the neutral and/or cationic hydroxo copper complexes are responsible for 60-70% of the toxicity in this set of experiments.

The limitations of this analysis are discussed and a set of experiments which should provide more definitive answers to the relative toxicity of these species is described.

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